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Mycorrhizal Inoculation of Container Grown Engelmann Spruce and Lodgepole Pine

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Forest Insect and Disease Management
State and Private Forestry
Rocky Mountain Region
Forest Service
U.S. Department of Agriculture

ABSTRACT

Partially decomposed sawdust, *Pisolithus tinctorius* (Pers.) Coker & Couch (strain 145) (Pt) vegetative mycelium, and forest duff were compared for effectiveness as ectomycorrhizal inocula for container-grown Engelmann spruce and lodgepole pine. Mixtures of sawdust with Pt and forest duff were also compared for effectiveness as inocula. Inoculations were made at seeding and at four months. Engelmann spruce grown in large ("jumbo") and small ("pine") Ray Leach tubes and inoculated at four months were compared for mycorrhizal development.

For Engelmann spruce, more mycorrhizal development resulted from natural inoculation than from the treatments. There was little natural inoculation of lodgepole pine, and all sources of inocula were ineffective. None of the mycorrhizae observed had the characteristic appearance of Pt ectomycorrhizae. The Pt strain 145 was isolated from oak and was ineffective as ectomycorrhizal inoculum in a previous experiment (warx 1977c). The sawdust apparently did not enhance mycorrhizal development. Spruce grown in larger containers showed more mycorrhizal development than those grown in smaller containers.

MYCORRHIZAL INOCULATION OF CONTAINER-GROWN ENGELMANN SPRUCE AND LODGEPOLE PINE

by

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INTRODUCTION

A mycorrhiza ("fungus-root") is a mutually beneficial symbiosis between a fungus and a root. In an ectomycorrhiza, fungal hyphae grow between the living primary cortical cells of the root, replacing the middle lamella, forming the Hartig net. A tightly interwoven mass of hyphae surrounds the root, forming the fungus mantle. Ectomycorrhizae are formed by ectomycorrhizal fungi with some angiosperms and gymnosperms, and are necessary for the proper growth and survival of most species of the Pinaceae (Marx 1975).

Ectomycorrhizae benefit plants through (1) increased absorptive surface of the feeder root system, especially by hyphae emanating into the soil; 2) more rapid absorption and accumulation of essential elements, especially nitrogen, phosphorous, potassium, and calcium; 3) increased longevity (functional life) of feeder roots; 4) increased availability of normally non-available nutrients from soil minerals; 5) increased tolerance to soil toxins, extreme soil acidity, drought, and high soil temperatures; and 6) added protection from root pathogens such as *Phytophthora* and *Pythium* spp. (Marx 1969a, 1969b, 1972, 1973). Mycorrhizae increase the growth and biomass of trees and other crops as measured by height, weight, and caliper (Marx 1977a). Fumigation for weeds, nematodes, and fungal pathogens can destroy or seriously deplete mycorrhizal fungal population levels in nursery seed beds (Marx 1972). Re-colonization with mycorrhizal fungi can occur through propagules carried in irrigation water or from neighboring infested beds by soil equipment, and by airborne spores from fruiting bodies in adjacent forests. Spore production and dissemination depend on the weather; hence, natural inoculation is unpredictable. Natural inoculation of shortleaf pine (*Pinus echinata* Mill.) in fumigated beds resulted in 90% mycorrhizal infection according to Marx and Bryan (1969; cited by Bowen and Theodorou 1973); whereas Wright (1971) found natural inoculation highly sporadic within beds of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco).

Natural inoculation of container-grown seedlings is even more erratic because of the sterilized potting soil and restrictive greenhouse conditions. High nutrient levels, especially of nitrogen and phosphorous, retard mycorrhizal formation (Brix and van den Driessche 1974, Voigt 1969). Natural inoculation by mycorrhizal fungi can occur, but infections are often sparse or absent (Peterson 1974). Sometimes natural inoculation of containerized seedlings is considerable, e.g. 55% of short roots were mycorrhizal in limber pine (*Pinus flexilis* James) and 77% of

short roots were mycorrhizal in lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) in Fort Collins, Colorado (Reid and Grossnickle 1978).

Non-mycorrhizal tree seedlings outplanted on forested sites eventually become infected with endemic mycorrhizal fungi, but there is a lag period in tree growth while infection takes place. Consequently, non-mycorrhizal trees exhibit poor survival after outplanting (Mikola 1973). Differences in growth rate between inoculated and non-inoculated seedlings are noticeable for 2.5 to 4 years after outplanting (Mikola 1973). On treeless sites far from forest (e.g. the Great Plains) and sites with adverse conditions, (e.g. mine spoils), outplanted seedlings must be mycorrhizal to survive. Also, if the mycorrhizal fungus cannot adapt to the adverse conditions, the tree dies. On forested sites, better-adapted indigenous mycorrhizal fungi gradually replace any inferior ones brought from the nursery, with minimal lag in tree growth (Mikola 1973).

Soil, duff, and transplanted mycorrhizal "mother" trees have been used in nursery beds as sources of mycorrhizal inocula (Mikola 1973). Soil inoculum is heavy, bulky, and the large amounts necessary (10% of volume of soil mix) are not easily transported (Mikola 1973). Soil may contain pathogens and weed seeds. Forest duff is readily available, but may also contain pathogens as well as beneficial fungi. Transplanting "mother" trees into nursery beds creates logistic problems during planting, and results in uneven infection in the nursery beds. Pure culture inoculations are often effective, but have required much hand labor.

Pisolithus tinctorius (Pers.) Coker & Couch (Pt) grows well in adverse sites and forms ectomycorrhizae with many tree species, including 30 species of *Pinus* (Marx 1972, 1977 b). Mass production of Pt vegetative mycelium for nursery inoculation is currently being developed by Abbott Laboratories, Long Grove, Illinois, in cooperation with the U. S. Dep. Agric., Forest Service, Mycorrhizal Research Institute, Athens, Georgia.

Partially-decomposed Engelmann spruce sawdust from an abandoned sawmill site on the Colorado State Forest is used to improve the texture of the potting soil by the Colorado State Forest Service Nursery in Fort Collins. The old sawdust was suspected as mycorrhizal inoculum when conifer seedlings growing in tar pots with the sawdust and soil mixture in Fort Collins became heavily infected with ectomycorrhizae (Landis 1976, personal communication). Fungal hyphae with clamp-connections were abundant in the decayed sawdust.

OBJECTIVES

The objective of this evaluation was to compare old spruce sawdust, Pt vegetative mycelium, forest duff, and mixtures of sawdust/duff and sawdust/Pt, as ectomycorrhizal inocula for container-grown Engelmann spruce and lodgepole pine. Mixtures of sawdust with other inocula were used to test whether sawdust enhanced mycorrhizal development. Two inoculation times, at seeding and at four months, and two container sizes, Ray Leach (RL) "jumbo" and RL "pine", were compared.

MATERIALS AND METHODS

INOCULATION

Spruce sawdust was collected from the abandoned sawmill site on the north fork of the Michigan River on the Colorado State Forest in May and August 1977. Spruce duff was collected to about 15 cm depth from a stand of Engelmann spruce above Ruedi Reservoir (about 30 miles southeast of the Mt. Sopris Nursery, Carbondale, Colorado) in June 1977, and from a spruce forest above Aspen, Colorado (30 miles south of the Mt. Sopris Nursery) in August 1977. Lodgepole pine duff was collected from the Colorado State Forest in May 1977. Inocula not used immediately were kept in cold storage for later use. Both the duff and the sawdust were sieved through a 5 mm wire mesh to remove large particles (Fig. 1).

Cultures of Pt strain 145, isolated and identified by D. H. Marx, were obtained from the Department of Forestry, Colorado State University, Fort Collins. Disks of mycelium were transferred to Modified-Melin-Norkrans (MMN) (Marx 1969 b, with glucose instead of sucrose) agar plates and allowed to grow 3 weeks at room temperature.

Vegetative mycelium was produced by the method of Marx and Bryan (1975) in two liter canning jars filled with 1500 ml vermiculite, 1000 ml MMN liquid medium, and 100 ml peat, autoclaved for 1 hour at 121° C. Disks (7 mm diameter) were cut from Pt mycelial mats growing on agar. Each jar was inoculated aseptically with at least 30 disks which were distributed in the upper 2/3 of the substrate with a long-handled spoon. Vegetative mycelium permeated the substrate in 4 weeks (Fig. 2).

The Pt inoculum was prepared by holding the jar contents in several layers of cheesecloth while leaching away the nutrient medium with cold running water (Fig. 3). Leaching removed excess nutrients which encourage the growth of damping-off fungi and other competing microorganisms. Pt was reisolated from the jars to confirm identity. Four jars of vegetative mycelium yielded approximately 4 liters of inoculum.

Inoculum was applied as a top-dressing to four-month-old containerized seedlings. Preliminary data showed top dressings might be more effective than soil mixes for ponderosa pine (*Pinus ponderosa* Laws.) (Landis 1977, unpublished data). The inoculation and evaluation schedule is presented in Table 1.

Fig. 1 Sieving duff through screen to remove cones and other large particles.

Fig. 2 Jars of Pt vegetative mycelium in peat, vermiculite, and MMN nutrient medium after four weeks of growth.

Fig. 3 Leaching away excess nutrient medium from Pt inoculum (held in cheesecloth).

1

2

3

TABLE 1. Inoculation and Evaluation Schedule

Inoculation designation	ES-4-June-Jumbo	ES-0-Aug-Small	LPP-0-Aug-Small	ES-4-Dec-Small	LPP-4-Dec-Small
Age at inoculation	Four months	At seeding	At seeding	Four months	Four months
Date of inoculation	June 30, 1977	Aug. 17-18, 1977	Aug. 17-18, 1977	Dec. 8, 1977	Dec. 8, 1977
Size of container	RL "jumbo"	RL "pine"	RL "pine"	RL "pine"	RL "pine"
Treatments (top dressings)	1. Sawdust 2. Spruce duff 3. Sawdust + duff mix 4. Control	On regular potting soil 1. Sawdust 2. Spruce duff 3. Pt 4. Control On sawdust+potting soil 5. Spruce duff 6. Pt 7. No top dressing	On regular potting soil 1. Sawdust 2. Pine duff 3. Pt 4. Control n sawdust+potting soil 5. Pine duff 6. Pt 7. No top dressing	1. Sawdust 2. Spruce duff 3. Sawdust + duff mix 4. Pt 5. Sawdust + Pt mix 6. Control	1. Sawdust 2. Pine duff 3. Sawdust + duff mix 4. Pt 5. Sawdust + Pt mix 6. Control
First evaluation	December 1977	January 1978	January 1978	May/June 1978	May/June 1978
Second evaluation	March 1978	May/June 1978	May/June 1978	-	-

ES-4-June-Jumbo

On June 30, 1977, four-month-old Engelmann spruce grown in Ray Leach (RL) "jumbo" tubes (4 cm top diameter by 21 cm length) were inoculated. Six trays (98 tubes/tray = 588 seedlings) were inoculated for each of 3 treatments: sawdust, spruce duff, and a 50% mixture of sawdust and duff. The standard greenhouse planting served as the control group. For each treatment, 8 liters of inoculum were hand-spread around the tops of the 6 containers (Fig. 4), then containers were gently sprayed with water. The seedlings had been moved from greenhouse to shadehouse in mid-June, and hardening-off was completed by September. This test was designated ES-4-June-Jumbo to indicate Engelmann spruce inoculated at four-months age in June, grown in RL "jumbo" tubes.

ES-0-Aug-Small and LPP-0-Aug-Small

On August 17-18, 1977, Engelmann spruce and lodgepole pine were sown in RL "pine" tubes (2.5 cm top diameter by 16.5 cm length). There were 7 treatments for each of the 2 tree species, with 3 trays (200 tubes/tray = 600 trees) per treatment. Each species had 4 groups (3 trays each) in regular potting soil (50% peat, 50% vermiculite, by volume) and 3 groups with 25% sawdust mixed with the potting soil. This is the mix rate used by the Colorado State Nursery. Approximately 8 liters of sawdust were used in each sawdust-soil mix group.

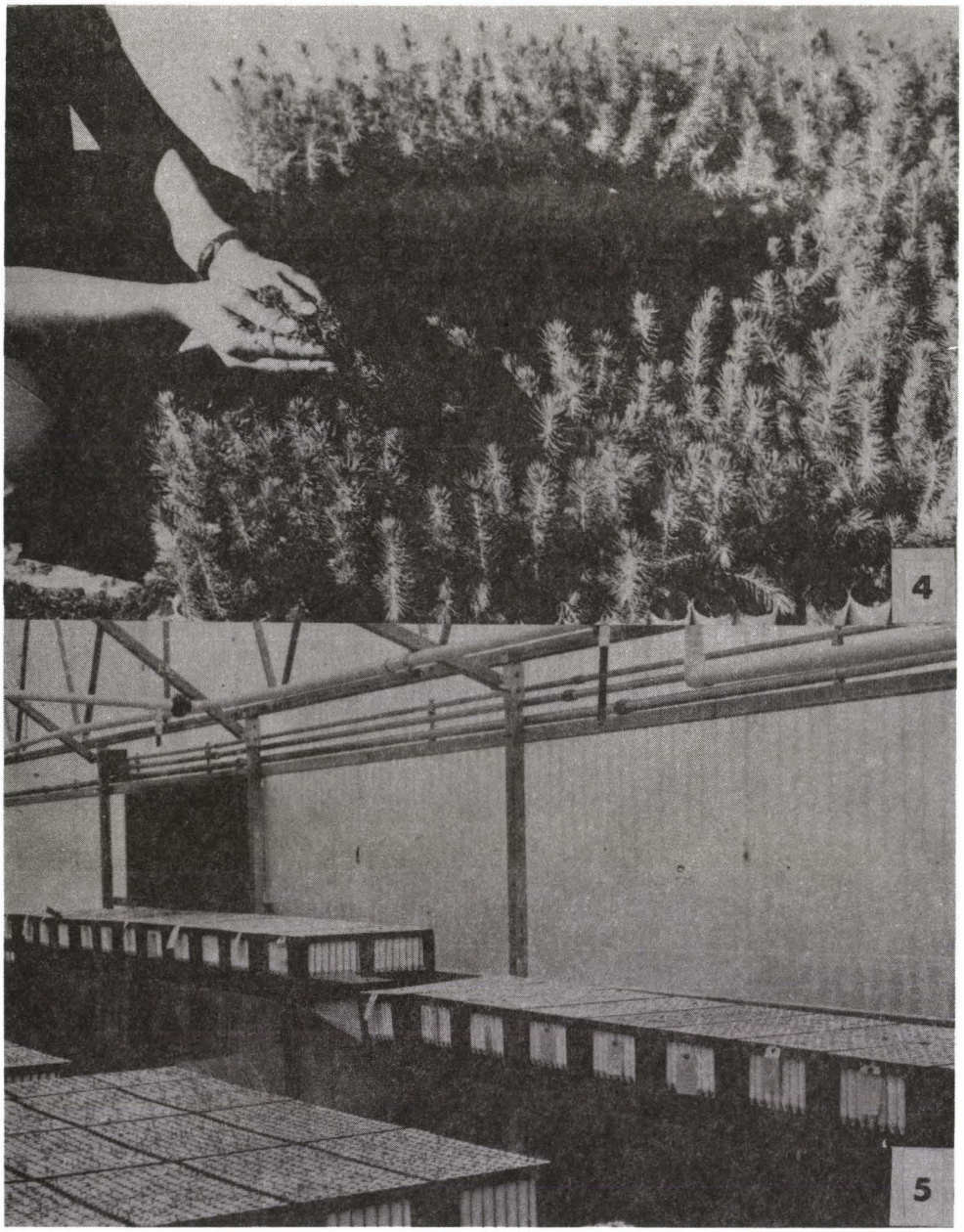
Of the 4 groups with regular potting soil, one group was left as a control, one received sawdust, one Pt top-dressing treatments. Of the three groups with sawdust-soil mix, one group was left without top-dressing, one received duff, and one Pt top-dressing treatments. Top-dressings consisted of 1-2 cm of inoculum placed on top of the soil in tubes and required about 4 liters of inoculum per three-tray treatment. The containers were seeded using standard templates, covered with perlite, watered, and placed along the north wall of the greenhouse (Fig. 5). The Engelmann spruce inoculated at seeding time in August in small containers were designated ES-0-Aug-Small, while the lodgepole pine were designated LPP-0-Aug-Small.

ES-4-Dec-Small and LPP-4-Dec-Small

On December 8, 1977, four-month-old Engelmann spruce and lodgepole pine seedlings in RL "pine" tubes were inoculated. These seedlings were from the standard greenhouse planting sown at the

Fig. 4 Spreading duff around tops of "jumbo"-size containers of four-month-old Engelmann spruce.

Fig. 5 Seeded trays (with tags) of the August-inoculated spruce and pine along the north wall of the greenhouse. The regular greenhouse planting is on the lower left.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beginning of August 1977. Three trays (600 seedlings) for each tree species were inoculated for each of 5 top-spread treatments: sawdust, duff, Pt, 50% sawdust-duff mix, and 50% sawdust and Pt mix. For each tree species, 3 trays of controls were separated from the regular stock and kept with the inoculated trays in the main part of the greenhouse. The Engelmann spruce inoculated at 4 months in December in small tubes were designated ES-4-Dec-Small, while the lodgepole pine were designated LPP-4-Dec-Small.

Environmental Regime

The standard growing regime for greenhouse container stock consists of sowing and germination of seed followed by 4 months of 21-24° C temperature, 50-70% R.H., watering at field capacity (1-3 bars), fertilization with a stock solution high in N (222 ppm) and low in P (60 ppm), and with supplemental night light at 45 f.c. for 1 min. every 30 min., in the greenhouse to maximize shoot growth. The spring crop (sown in late February) is moved to the shadehouse in June where the temperature and R.H. are ambient, watering is at field capacity (1-3 bars), fertilization is with a stock solution low in N (27 ppm) and high in P (150 ppm), and without supplemental light at night, to maximize root growth and increase caliper. As the ambient temperatures and photoperiod change the trees harden off gradually and when dormant (September) are watered to field capacity when dry (15 bars). The fall crop (sown in August) undergoes a change of conditions after the 4 months of shoot growth (December) to 16-18° C temperature, 30-70% R.H., watering at field capacity (1-3 bars), fertilization with a stock solution with low N and high P, and no supplemental light at night, to maximize root growth and increase caliper. By February the fall crop is dormant; the temperature is -1 to 2° C, and watering is to field capacity when dry (15 bars).

EVALUATION OF SEEDLINGS

In the first evaluation of ES-4-June-Jumbo inoculation, 120 trees were sampled. In subsequent evaluations of all inoculations, 60 trees were sampled from each treatment. The time between inoculation and first evaluation was approximately 5.5 months for all inoculations (Table 1).

Seedlings were rated for presence or absence of mycorrhizae by examination of the surfaces of the root wads with a stereoscope at 12X magnification. Sections of some mycorrhizae were viewed under a microscope for confirmation. The Heterogeneity Chi-Squared test was used to compare treatments for each inoculation time (Snedecor and Cochran 1967). The G statistic for proportions was used to compare results between inoculation times and between treatments across inoculation times (Sokal and Rohlf 1969). When comparisons were made across inoculation times, the G statistic for hetero--geneity was used as a test of independence. In this test, groups with zero values cannot be meaningfully compared with groups with other numerical values. The G tests are nonparametric tests which provide information similar to that derived from analysis of variance. The data were discontinous (either a tree was mycorrhizal or it was not) and consequently did not fit the assumptions of analysis of variance.

RESULTS

Results are presented in Tables 2 through 6, and are expressed as percentages of trees with mycorrhizae. Significance was tested at the 0.05 level. The first evaluation in January of the ES-0-Aug-Small and LPP-0-Aug-Small inoculations indicated no mycorrhizal development, so this evaluation was omitted from analysis. Landis and Gillman (1976) found that Pt did not infect containerized ponderosa pine until after seedling fertilization was reduced at the beginning of the hardening-off period. The January evaluation was made at the onset of the hardening-off period. Identification of the naturally occurring ectomycorrhizal fungal symbionts infecting spruce and pine in this study was not attempted.

Table 2 shows the non-significant sets of treatments for each inoculation, as determined by the Heterogeneity Chi-Squared test. With the exception of the sawdust and sawdust + duff mix treatments in the March evaluation of the ES-4-June-Jumbo inoculation, no treatment resulted in more mycorrhizae than that which developed from natural infection of the controls.

Table 3 compares the Engelmann spruce results for all common treatments using the G statistic for proportions. Since the ES-0-Aug-Small inoculation had two different sawdust alone treatments, the results are presented separately in Tables 3 A and 3 B. In both tables, comparison of the column totals shows that the duff treatment had significantly less mycorrhizal development than the control, sawdust, and sawdust + duff mix treatments. As seen by comparison of row totals the ES-4-June-Jumbo inoculations had more mycorrhizal development than the two inoculations in smaller tubes. As shown by comparison of row totals in Table 3 A (sawdust-soil mix with no top-dressing treatment as the sawdust alone treatment for the ES-0-Aug-Small inoculation), the ES-0-Aug-Small and ES-4-Dec-Small results were not significantly different. But as shown in Table 3 B (regular potting soil with sawdust top-dressing treatment as sawdust alone treatment), the ES-4-Dec-Small inoculation had more mycorrhizal development than the ES-0-Aug-Small inoculations.

Table 4 compares all treatments for the August (at seeding) and December (at 4 months) Engelmann spruce inoculations using the G statistic for proportions. Tables 4 A and 4 B differ in the sawdust alone treatment for the ES-0-Aug-Small inoculation, and the results also differ. In Table 4 A, the row totals for the control and sawdust treatments were not significantly different. In Table 4 B, the row total for the control group was greater

TABLE 2. Non-significant Sets of Treatments Within Inoculation and Evaluation Times

ES-4-June-Jumbo
December evaluation

Percentage Mycorrhizal	Treatment
77	Control
62	Sawdust+duff mix
59	Sawdust
26	Duff

ES-4-June-Jumbo
March evaluation

Percentage Mycorrhizal	Treatment
78	Sawdust
75	Sawdust+duff mix
40	Duff
28	Control

ES-0-Aug-Small
May/June evaluation

Percentage Mycorrhizal	Treatment
30	Control
12	Potting soil+Pt
12	Sawdust soil,no top dressing
5	Potting soil+duff
5	Sawdust soil+duff
2	Potting soil+sawdust
2	Sawdust soil+Pt

ES-4-Dec-Small
May/June evaluation

Percentage Mycorrhizal	Treatment
27	Control
22	Sawdust
20	Sawdust+duff mix
15	Sawdust+Pt mix
10	Duff
8	Pt

LPP-0-Aug-Small
May/June evaluation

Percentage Mycorrhizal	Treatment
10	Sawdust soil+Pt
10	Potting soil+Pt
7	Potting soil+sawdust
5	Control
5	Sawdust soil+duff
5	Sawdust soil,no top dressing
0	Potting soil+duff

LPP-4-Dec-Small
May/June evaluation

Percentage Mycorrhizal	Treatment
10	Sawdust+duff mix
3	Sawdust
3	Sawdust+Pt
2	Pt
2	Control
0	Duff

Vertical lines indicate non-significant sets ($P>0.05$, with a-1 df where a=number of groups) as determined by the Heterogeneity Chi-Squared test.

TABLE 3. Comparison of Engelmann Spruce Results (percentages of trees with mycorrhizae) across all Inoculations and Evaluations

Inoculation	3 A[a] Control	h Sawdust	h Sawdust + duff	h Duff	Totals (df = 3)	3 B[b] Control	h Sawdust	h Sawdust + duff	h Duff	Totals (df = 3)
ES-4-June-Jumbo Dec. evaluation	77	59	62	26	224	77	59	62	26	224
ES-4-June-Jumbo March evaluation	28	78	75	40	221	28	78	75	40	221
ES-0-Aug-Small	30	12	5	5	52	30	2	5	5	42
ES-4-Dec-Small	27	22	20	10	79	27	22	20	10	79
Totals (df = 3)	162	171	162	81		162	161	162	81	

a
3 A: Sawdust treatment for ES-0-Aug-Small is sawdust soil with no top dressing.

b
3 B: Sawdust treatment for ES-0-Aug-Small is regular soil with sawdust top dressing.

Lines indicate non-significant sets (P>0.05) as determined by the G statistic for proportions. For the results of the duff treatment, there are three non-significant sets: 5 and 10, 10 and 26, 26 and 40.

Homogeneous treatment sets (those whose results varied in the same way across inoculation times, as determined by the G test for heterogeneity), are indicated by "h" preceding the treatment name.

TABLE 4. Comparison of Engelmann Spruce Results (percentages of trees with mycorrhizae) for all Treatments Across the August and December Inoculations

Treatment	4 A[a] ES-0-Aug-Small	ES-4-Dec-Small	Totals (df = 5)	4 B[b] ES-0-Aug-Small	ES-4-Dec-Small	Totals (df = 5)
hh Control	30	27	57	30	27	57
h Sawdust	12	22	34	2	22	24
h Sawdust + duff	5	20	25	5	20	25
hh Pt	12	8	20	12	8	20
h Sawdust + Pt	2	15	17	2	15	17
h Duff	5	10	15	5	10	15
Totals (df = 1)	66	102		56	102	

a
4 A: Sawdust treatment for ES-0-Aug-Small is sawdust soil with no top dressing.

b
4 B: Sawdust treatment for ES-0-Aug-Small is regular soil with sawdust top dressing.

Lines indicate non-significant sets (P>0.05) as determined by the G statistic for proportions.

Homogeneous treatment sets (those whose results varied in the same way across inoculation times, as determined by the G test for heterogeneity) are indicated by a number of "h's" preceding the treatment name.

than that of all other treatments. As can be seen by comparison of the column totals, the December inoculation resulted in more mycorrhizal development than the August inoculation. This trend held for each treatment except for the control and Pt treatments, which were not significantly different between inoculations.

For the Engelmann spruce inoculations, overall, no treatment resulted in more mycorrhizal development than that from natural infection of the controls. Other workers have reported difficulty in inducing mycorrhizal development on Engelmann spruce in the greenhouse, even in experiments where fertilization was reduced to stimulate mycorrhizal development (Reid and Grossnickle 1978).

Table 5 compares all treatments for the August and December lodgepole pine inoculations using the G statistic for proportions. Tables 5 A and 5 B differ in the sawdust alone treatment for the LPP-O-Aug-Small inoculation, but the results differ only slightly between the two tables. The August inoculation had more mycorrhizal development than the December inoculation, as shown by comparison of column totals. There was little natural inoculation in the control groups for pine, but no treatment gave significantly better results than the controls, as shown by comparison between the row totals for each treatment.

Table 6 compares the results for the Engelmann spruce and lodgepole pine inoculations using the G statistic for proportions. Table 6 A compares the August inoculations, and Table 6 B compares the December inoculations. For both sets of inoculations the overall mycorrhizal development (as shown by the column totals) for the Engelmann spruce was better than that for the lodgepole pine. In the December inoculations, spruce results were better than the pine for each treatment. In the August inoculations, spruce results were better than the pine in the control, sawdust soil alone, and duff alone treatments. Pine results were better than the spruce in the sawdust soil + Pt and sawdust top-dressing treatments for the August inoculations.

DISCUSSION AND CONCLUSIONS

Artificial inoculations of container-grown Engelmann spruce and lodgepole pine with sawdust, Pt strain 145, and forest duff were ineffective in inducing the formation of ectomycorrhizae.

TABLE 5. Comparison of Lodgepole Pine Results (percentages of trees with mycorrhizae) for all Treatments across the August and December Inoculations

Treatment	5 A[a] LPP-0-Aug-Small	LPP-4-Dec-Small	Totals (df = 5)	5 B[b] LPP-0-Aug-Small	LPP-4-Dec-Small	Totals (df = 5)
Sawdust + duff	5	10	15	5	10	15
Sawdust + duff						
h Sawdust + Pt	10	3	13	10	3	13
h Pt	10	2	12	10	2	12
h Sawdust	5	3	8	7	3	10
h Control	5	2	7	5	2	7
Duff	0	0	0	0	0	0
Totals (df = 1)	35	20		37	20	

[a] 5 A: Sawdust treatment for LPP-0-Aug-Small is sawdust soil with no top dressing.

[b] 5 B: Sawdust treatment for LPP-0-Aug-Small is regular soil with sawdust top dressing.

Lines indicate non-significant sets (P>0.05) as determined by the G statistic for proportions.

Homogeneous treatment sets (those whose results varied in the same way across inoculation times, as determined by the G test for heterogeneity) are indicated by "h" preceding the treatment name.

TABLE 6 . Comparison of Engelmann Spruce and Lodgepole Pine Results (percentages of trees with mycorrhizae) for the August (6 A) and December (6 B) Inoculations

6 A	Treatment	ES-0-Aug-Small	LPP-0-Aug-Small	Totals*
	Control	30	5	35
h	Potting soil + Pt	12	10	22
h	Sawdust soil,no top dressing	12	5	17
hh	Sawdust soil + Pt	2	10	12
h hh	Sawdust soil + duff	5	5	10
hh	Potting soil + sawdust	2	7	9
h	Potting soil + duff	5	0	5
	Totals(df=1)	68	42	

6 B	Treatment	ES-4-Dec-Small	LPP-4-Dec-Small	Totals*
h	Control	27	2	29
hh	Sawdust+duff mix	20	10	30
h	Sawdust	22	3	25
h	Sawdust+Pt mix	15	3	18
hh	Pt	8	2	10
h	Duff	10	0	10
	Totals(df=1)	102	20	

*Totals for each treatment across both inoculations were not analyzed statistically.
Lines indicate non-significant sets ($P>0.05$) as determined by the G statistic for proportions.
Homogeneous treatment sets (those whose results varied in the same way across inoculation times, as determined by the G test for heterogeneity) are indicated by a number of "h's" preceding the treatment name.

The disparity between the two evaluations of the June-inoculated Engelmann spruce, in which the infection in the controls was 77% (the highest) in December and 28% (the least) the following March (Table 2), is most likely due to sampling error. Controls for the December evaluation evidently received heavy natural mycorrhizal infection. For the March evaluation only a few trays of the regular spruce planting remained in the shadehouse since most had been outplanted. These remaining seedlings evidently received little natural infection. This illustrates the sporadic nature of natural inoculation by mycorrhizal fungi in greenhouse and shadehouse environments.

The August-inoculated Engelmann spruce and lodgepole pine were intended to receive the same care in the greenhouse as the regular August plantings, but the inoculated seedlings showed markedly reduced growth and survival several months later. This difference was attributed to their position (along the wall) in the greenhouse, where fertilization, watering, and ventilation conditions must have been less than optimum.

The RL "jumbo" tubes used for the June inoculation have 2.5 times the top surface area and over 3 times the volume of the RL "pine" tubes used for the August and December inoculations. The advantages of the larger tube are that more nutrients and water can enter because of the larger top surface area, and there is more space for water storage and root growth in the greater volume. The advantage of the smaller tube is that 200 trees can be grown in the space required by 98 trees in the larger tubes. After several months growth, especially in the smaller tubes, tree seedling tops are bushy enough to block the path of water and fertilizer to the potting medium. This was evident when root wads were still dry after several rainy days in May.

The greater surface area of the "jumbo" tube may increase the chance for natural inoculation by airborne spores of mycorrhizal fungi. Also more spores may have drifted in during summer and fall when the June-inoculated spruce were outside in the shadehouse. In contrast, the August- and December-inoculated spruce were outside in the shadehouse in winter and early spring, and showed much less mycorrhizal development.

The growing conditions along the wall in the greenhouse may have been the reason for the poor results obtained in the August-inoculated spruce compared to the December. The decreased mycorrhizal development may have been due to inhibited root growth from excessive soil-packing from tamping down the top-dressings in the soil-filled containers for the spruce inoculated at seeding time in August.

The lodgepole pine inoculated in August and grown along the wall in the greenhouse developed more mycorrhizae than the December-inoculated pine. The August-inoculated pine, since they were placed along the wall, may have received less fertilization, which may have been the reason for the better mycorrhizal development. The difference cannot be due to the amount of time allowed for mycorrhizal development, since both the August- and December-inoculated pine were sown in August and the treatments were not significantly different from the natural inoculation in the control groups.

Pt applied as vegetative mycelium in vermiculite has been an effective inoculum for many trees, especially pines, not only in the southeast (Marx 1975), but also in Colorado (Landis and Gillman 1976; Landis 1977, unpublished data; Reid and Grossnickle 1978). In this experiment, Pt strain 145 did not infect lodgepole pine or Engelmann spruce. None of the mycorrhizae on the spruce or pine treated with Pt inoculum exhibited the characteristic Pt appearance. It was later learned that the Pt isolate used in this study was isolated from oak and had failed to infect trees in a previous experiment (Marx 1977c).

In conclusion, the partially decomposed sawdust, Pt strain 145, and forest duff were ineffective as ectomycorrhizal inoculum for container-grown Engelmann spruce and lodgepole pine. Sawdust apparently did not enhance mycorrhizal development. For Engelmann spruce, mycorrhizal development resulting from natural inoculation in the control groups was the same or better than the development in any of the treated groups. Spruce sown in the spring and grown in large RL "jumbo" containers had better mycorrhizal development than spruce sown in August and grown in small RL "pine" containers. For lodgepole pine, mycorrhizal development resulting from natural inoculation in the control group was poor, but no less than that which developed in the treated groups. Whether another strain of Pt can infect Engelmann spruce remains to be evaluated. Since the artificial inoculations in this study were ineffective, no conclusions can be drawn regarding the timing of inoculation.

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